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A STUDY OF PERMEABILITY BY THE METHOD OF TISSUE TENSION

S. C. Brooks

A number of investigators have shown that recovery from plasmolysis is not wholly satisfactory as a criterion of the permeability of protoplasm to a plasmolyzing agent because of the possible injurious mechanical effect of the plasmolysis itself.[1] It is evident that this difficulty disappears if instead of plasmolysis and recovery we use as a criterion the shrinkage and the elongation of young, highly stretched tissues, taking care that the shrinkage does not proceed far enough to cause a retraction of the protoplasm from the cell walls. This is most conveniently done with tissues in which the changes in turgidity are indicated by a bending of the tissue, due to great differences in the elasticity of the cell wall in its different layers.

In rapidly elongating plant tissues there is usually a very considerable pressure exerted by the protoplasts against the cell walls which confine them. If all the cell walls of the stem are thin and elastic, the whole stem will be kept in a stretched condition by this pressure. The presence of thick walled cells, such as fibro-vascular or epidermal cells, which are not easily stretched by internal pressure, will, if they are symmetrically distributed, prevent this elongation of the tissue. If now we cut such a stem or peduncle in such a way that these two types of tissue are unsymmetrically distributed, the whole tissue will curl so that the elastic tissue forms the longer, or convex side. The distension of the elastic tissues, and therefore the degree of curvature, will vary with the turgidity of the tissues. A hypertonic solution will withdraw water from the cells, and consequently reduce the turgidity and the degree of curvature, while a hypotonic solution will have the opposite effect. The penetration of the protoplasm by a salt with whose solution such a tissue had come into osmotic equilibrium would lead to an increase in the turgidity, and hence in the curvature of the tissue. De Vries (8), in the investigation of the isotonic coefficients of various substances by this method, observed such

[1] Cf. Bower (1), Chodat and Boubier (3), Hecht (4), and Küster (5).

a secondary increase in the curvature of strips from the peduncles of *Centranthus ruber* and *Rudbeckia triloba.*

Among such tissues, strips of peduncles of the dandelion (*Taraxacum officinale* Weber) are well known for their large and rapid response to changes in the concentration of the solution in which they are immersed. Such strips also showed themselves to be excellent material for the study of the rate of penetration of salts. Upon being cut, they are forced by the existing tension to bend rather strongly outward around an axis tangential to the peduncle. If they are then placed in a slightly hypertonic solution, their curvature decreases during a period varying from a few seconds up to several minutes, remains constant a moment and then slowly increases, sooner or later exceeding the original curvature. The last phase is analogous to the recovery of plasmolyzed cells, and will, for convenience, be also termed "recovery."

By means of observations on the rate at which this recovery occurred in various salt solutions it was possible to determine the permeability of dandelion protoplasm to inorganic salts, and the progressive changes in permeability produced by such salts.

METHOD

The salts used were the same or of the same grade of purity as those used in the experiments on exosmosis from dandelion tissue, as described by the writer in a recent paper (2). They were dissolved in distilled water which had a specific conductivity of less than 2×10^{-6} ohms. Molecular stock solutions were made up and carefully standardized by titration against a 0.1 *M* solution of silver nitrate with potassium monochromate as an indicator. The desired concentrations were secured by dilution of these stock solutions, and were accurate to 0.5 percent. This accuracy was sufficient for the purposes of the experiment.

Strips of dandelion peduncle, each about 2.5 cm. long and 3 mm. wide, or similar strips from the midrib of the leaf were used in these experiments.[2] There was practically no difference in the data furnished by the two types of tissue.

Each strip was firmly gripped at one end by the two halves of a partially split rubber stopper, which in turn was secured by means of

[2] The midrib, like the peduncle, is hollow and for the sake of uniformity strips from the upper (ventral) half of this tissue were always used.

de Khotinsky's cement to the bottom of a glass dish 6.5 cm. in diameter and 1.5 cm. deep. The strips were held horizontally in such a position that as the free end bent it moved back and forth horizontally.

Immediately after the strips of peduncle were cut, they were put in place in the glass dish and covered with 20 cc. of an isotonic solution of the salt to be investigated. The dish was then covered with a glass plate and set on the stage of a microscope. The glass plate was pierced by an opening just large enough to allow the introduction of the front of the Bausch and Lomb 2/3 inch objective, by means of which, in combination with a No. 7.5 ocular, provided with an ocular

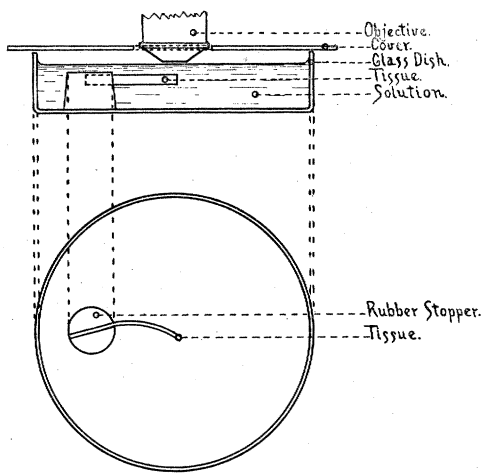

FIG. 1.

micrometer, the position and rate of movement of the free end of the strip was determined. Figure 1 shows the arrangement of the apparatus.

The epidermal surface of the strip furnished a sharply defined point of reference. The evaporation from a dish so covered, with the objective in place, amounted to about 0.05 percent per hour at room temperature; this amount was not sufficient to have any appreciable effect on the time required for recovery.

A solution was considered to be isotonic with the cells of the tissue when there was a barely perceptible decrease in the curvature of the strips of peduncle immediately following their immersion in the solution. This condition signified that the solution was actually

slightly hypertonic to the cells; but a very slight shrinkage of the cells, such as caused the decrease in curvature of the tissue, sufficed to raise their osmotic pressure to equality with that of the solution, and recovery proceeded as rapidly as the rate of penetration of the plasmolyzing substance would permit. It will be seen that while the solutions used were not strictly isotonic with the cells, the latter adjusted themselves to the solution, becoming isotonic with it within at most a very few minutes.

As soon as a condition of isotony was established between cells and solution, the concentration of the latter was increased by the addition of a measured small amount of a molecular solution of the salt, and the solution quickly stirred in order to secure a uniform distribution of the increase in concentration. In order to avoid very great differences in the amount of shrinkage, such as might cause a mechanical alteration of the cell walls, or injury to the protoplasm, it was necessary to vary this increase in concentration according to the salt used. Thus, for the sea water-calcium chloride mixture, described by the writer in a previous paper (2), and for the salts of univalent kations 0.2 cc. of 1 *M* solution was added to 20 cc. of the isotonic solution which had a concentration of 0.20 to 0.235 *M*. In the case of bivalent kations the concentrations were 0.15 to 0.17 *M* and to 20 cc. of the solution there was added 0.1 cc. of a 1 *M* solution to produce the desired increase of concentration. In the case of trivalent kations the concentrations were .045 to .065 *M* and 0.05 cc. of a 1 *M* solution was added when it was desired to increase the concentration. The maximum error in making these changes was 0.0001 *M*.

This increase in concentration resulted in a decrease in the curvature of the strip of tissue which soon ceased and was followed by a slower movement in the opposite direction. The time elapsing between the increase of concentration and the moment when the strip regained its original curvature (*i. e.*, returned to its initial position on the scale of the ocular micrometer) was recorded as the "time of recovery." Immediately upon the recovery of a given strip, the concentration of the solution bathing it was again raised by the same amount as before, and the time of recovery again noted. By repetition of this process we secure a series of recoveries of one strip of tissue, the time required for each recovery being a measure of the average rate of penetration of salt during that recovery, and the initial and final curvature being always the same throughout the whole of the experiment.

In order to obtain figures for the different groups of salts which would be comparable with one another, it was necessary to correct for the difference between the concentration changes used for these groups. An empirical expression of the rate of penetration of a salt may be obtained by dividing the concentration change causing the decrease in curvature by the time in minutes required to regain the initial curvature, or, as we have termed it, the "recovery time."

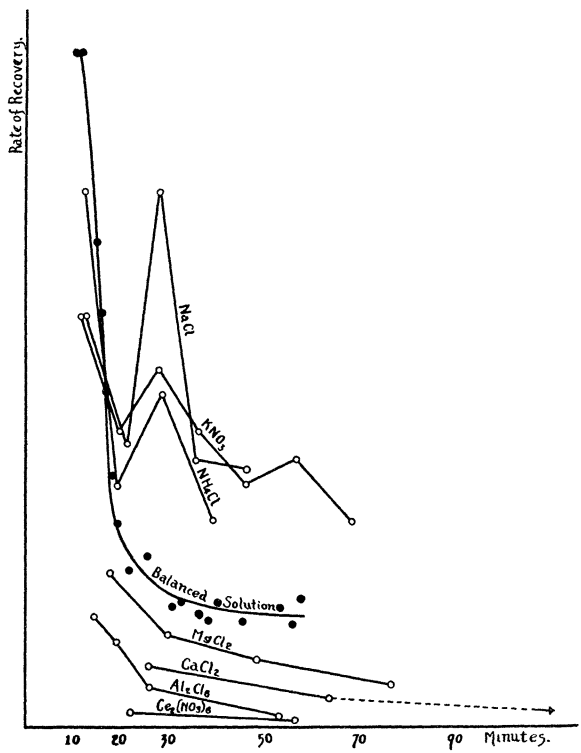

FIG. 2

If such figures be used as the ordinates, and as abscissae the time elapsed between the first immersion of the tissue in the solution and the middle of the recovery time, there will be produced a curve expressing the permeability of the cells after any given period of exposure to the action of a salt. Such curves are shown in Figure 2.

RESULTS

It was found that in all the solutions the rate of recovery decreased rapidly during the first fifteen or twenty minutes, and that all the experiments, with the exception of those with the sea water—calcium chloride solution, gave data which were quite erratic during that period. An examination of the curve (Fig. 2) representing the rate of penetration of this mixed solution will show that the points, plotted from four experiments, fall very uniformly on a smooth curve which shows a rapid decrease in the rate of recovery during the first twenty-five minutes, after which the rate remains nearly constant. This decrease in the rate of recovery represents the effect of some factor whose nature has yet to be determined, and which is common to all the salts used. In view of the presence of this factor and of the erratic behavior of the tissue during the first one or two recoveries in solutions other than the sea water-calcium chloride mixture, it seems best, for the present, to disregard the first twenty minutes of these experiments.

TABLE I

Time Required for Successive Recoveries, Taraxacum

Kations	Solution	Concentration	Increase of Concentration	Minutes in Solution Before 1st Recovery	Recovery Time, Minutes						
					1st	2nd	3d	4th	5th	6th	7th
Balanced	Sea water and $CaCl_2$ Mixture	0.235	0.0075	9	4	13	25	—	—	—	—
				10	4	16	21	—	—	—	—
				10	2.5	5.5	15.5	25	—	—	—
				13	10.5	24	21	—	—	—	—
				13	8	21.5	22	—	—	—	—
				13	6.5	23.5	26	—	—	—	—
Univalent	NaCl	0.22	0.0075	10	6.5	9.5	5.0	10	10.5	—	—
	KNO_3	0.21	0.0075	9	6.5	9.0	7.5	9.0	11.0	10.0	13.0
	NH_4CL	0.23	0.0075	6	3	5	11	8	13	—	—
Bivalent	$CaCl_2$	0.165	0.0041	14	24	51	83	—	—	—	—
	$MgCl_2$	0.15	0.0041	13	9.5	15	22	34	—	—	—
Trivalent	$Ce_2(NO_3)_6$	0.065	0.0011	9	26	43	—	—	—	—	—
	Al_2Cl_6	0.047	0.0011	13	3.5	4.5	9.5	—	45	—	—

In the experiments with solutions of pure salts the time at which characteristic alteration of permeability occurred varied sufficiently with different peduncles and even with adjacent strips from the same

peduncle, to make it ordinarily impossible to construct a composite curve which should fairly represent the characteristic effect of the salt. Therefore experiments have been selected which represent as nearly as possible the mean of all the experiments with the same salt, and the curves representing the progressive changes in rate of recovery plotted in Figure 2. The original data of these experiments are given in Table 1.

The difference in the behavior of the three groups of kations employed is very striking. The curves for sodium, potassium and ammonium salts lie everywhere above those for the mixed solution; the rate of recovery, and therefore the rate of penetration of the salt, is and remains greater than that normal for the protoplasm. The rate of penetration of calcium and magnesium salts is considerably, and that of salts of the trivalent kations (cerium and aluminium) very much below the normal.[4] It is to be noticed that sodium, potassium, and ammonium salts cause a marked increase in the rate of recovery between the twentieth and thirtieth minutes.

The secondary decrease, in the light of the experiments on the influence of salts on exosmosis, is probably to be attributed to the fact that the increase of permeability leads to a considerable rate of exosmosis, thus retarding the increase of the intracellular osmotic pressure, and hence decreasing the rate of recovery. It will be seen that sodium and ammonium chlorides, the most toxic among the three salts of univalent kations, cause the most rapid secondary fall of the curve, while potassium nitrate, the least toxic, causes only a slight fall. This fact also favors the supposition that the cause of secondary retardation in the rate of recovery is to be sought in an increase rather than a decrease of permeability; but this explanation must remain purely hypothetical pending the accumulation of further evidence with respect to the phenomenon. These experiments are thus found to be in essential agreement with those of Osterhout in which *Laminaria* was the plant used, although the fluctuations in permeability induced by pure salts were greater and more rapid than those in *Laminaria*. The

[3] The first recovery times are, however, usually in the same sequence in a series of salts as are the later recoveries.

[4] These curves were probably not disturbed by differences in the acidity of the solutions, except possibly in the case of cerium and aluminium chlorides, whose hydrogen ion concentrations, as determined by the use of a hydrogen electrode, were $3 \times 10^{-4}M$ and $4 \times 10^{-4}M$ at the concentrations used.

writer's experiments on *Laminaria*, by a diffusion method, are also in essential agreement with these experiments.[5]

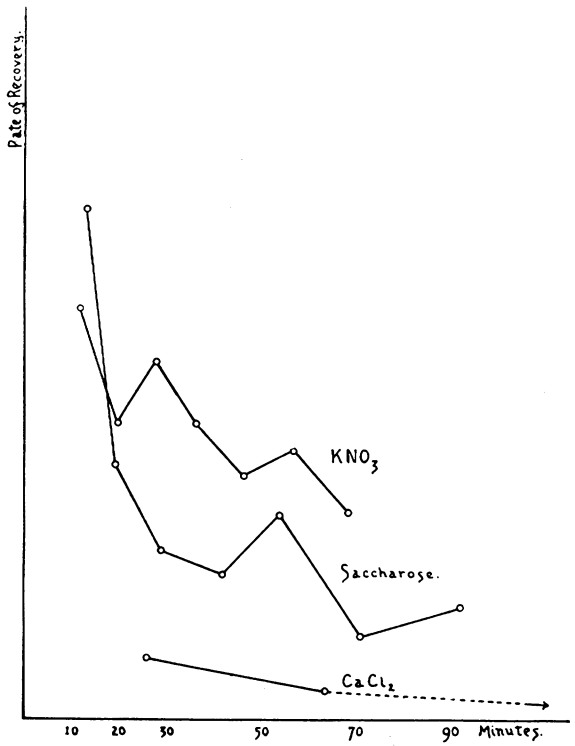

FIG. 3

TABLE 2

Time Required for Successive Recoveries, Taraxacum

Solution	Concentration	Increase of Concentration	Minutes in Solution Before 1st Recovery	Recovery Time, Minutes						
				1st	2d	3d	4th	5th	6th	7th
KNO_3.....	0.21	0.0075	9	6.5	9.0	7.5	9.0	11.0	10.0	13.0
$CaCl_2$.....	0.165	0.0041	14	24	51	83	—	—	—	—
Saccharose .	0.35	0.0064	11	5	8	12	14	10	24	18

In view of the widespread assumption that protoplasm is in general impermeable to saccharose, and that cells may be kept in solutions of saccharose without suffering any change in permeability,[6] the data of

[5] In process of publication.

[6] Cf. Lepsechkin (6).

experiments on the rate of recovery in saccharose solutions are of considerable importance. In Table 2 and Figure 3 are presented the data of a characteristic experiment with saccharose, together with those of similar experiments with potassium nitrate and calcium chloride for purposes of comparison. Saccharose affects the permeability of the protoplasm in the manner typical of a salt of a monovalent kation, but the changes occur with less rapidity. It is therefore unsafe to use saccharose as an indifferent medium; a properly balanced mixture of salts should rather be used when a solution is desired in which to maintain the normal permeability of living cells, as is evident from a comparison of Figures 2 and 3, disregarding (as previously explained) the first 20 minutes of the experiment.

SUMMARY

1. The permeability of the protoplasm of *Taraxacum officinale* Weber remains nearly or quite normal in a balanced solution consisting of a mixture of sea water and calcium chloride, such that the ratio of univalent to bivalent kations is approximately seventy to fifteen.

2. Salts of univalent kations in pure solutions cause a rapid increase in permeability.

3. Salts of bi- and trivalent kations cause a very great decrease in permeability.

4. Saccharose penetrates protoplasm quite rapidly, and affects permeability like a univalent kation.

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